Helsinki, 31 August 2022
RAC/62/2022/05
Agreed

62ND MEETING OF THE COMMITTEE FOR RISK ASSESSMENT

12-15 September 2022

Face-to-face meeting[1]

Concerns:	**RAC Guidance Note:**
	Addressing developmental neurotoxicity and neurotoxicity under the current CLP hazard classes
Agenda Point:	**8.1.3**
Action requested:	**For discussion/agreement**

This document was presented at RAC-61, after which it was updated based on the discussion. Comments were received from eight RAC Members during the written consultation carried out from 15 July to 12 August. The document was then revised further and following legal scrutiny was presented to RAC for agreement.

[1] Members are expected to attend in person.

Hazard classes to address developmental neurotoxicity (DNT) and neurotoxicity

It has been a matter for debate whether developmental neurotoxicity should be assessed and concluded under reproductive toxicity (developmental toxicity) or under specific target organ toxicity – single or repeated exposure (STOT SE or RE), especially when there is evidence that the nervous system is also a target organ following adult exposure. It is however important to have an aligned approach, not only between different substances within one regulatory process, but also between different regulatory processes for the same substance.

Authorities should be able to predict the regulatory value of developmental neurotoxicity studies when e.g. requesting new studies (such as OECD TG 443 with DNT cohorts 2A and 2B or OECD TG 426), when proposing regulatory strategies for substances. Industry faced with self-classification and dossier submitters need to know under which hazard class to address DNT. Reproductive toxicity is also a priority hazard class for harmonised classification and labelling (CLH) unlike STOT SE or RE (CLP Article 36.

A paper on the topic prepared by ECHA was circulated prior to the 61st meeting of the Committee for Risk Assessment, during which ECHA gave a presentation a proposing a standard approach. A revised version was consulted with RAC from 15 July to 12 August 2022. Eight RAC members provided written comments and the current version includes the amendments and revisions proposed by RAC members during the consultation and subsequently finalised following discussion at RAC-62.

Some parts of it may be proposed for inclusion in a future revision of the CLP guidance and potentially also in other ECHA guidance documents.

Approach

According to this approach:

1. Adverse effects on the nervous system investigated or detected at any point in the life span of the organism exposed during the developmental period, covering both prenatal and postnatal development until sexual maturation (determined by preputial separation and vaginal opening), should be addressed under developmental toxicity (DNT), even if the exposure had also continued after sexual maturation.
2. On the other hand, adverse effects on the nervous system caused by exposure of mature animals (exposure only after sexual maturation) should be addressed under STOT SE or STOT RE, depending on whether the effects are caused by single or repeated exposure, respectively.
3. If the CLP criteria for classification for STOT SE/RE **and** for reproductive (developmental) toxicity are met by data on neurotoxicity detected both after exposure occurring only after sexual maturation and after developmental exposure (even if the exposure had continued after sexual maturation and the neurotoxic

effect was detected at any point in the life span), respectively, then both classifications should apply.

It is noted that the CLP criteria for developmental toxicity and STOT SE/RE can also apply to other target organ toxicities. Developmental toxicity includes **any effect** which interferes with normal development, resulting from exposure of the developing offspring to the time of sexual maturation, such effects can be manifested at any point in the life span and include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency (CLP 3.7.1.4.). STOT SE and STOT RE cover specific target organ toxicities that are not specifically addressed in other human health hazard sections (CLP 3.8.1.1. and 3.9.1.1.). RAC highlighted that the same approach as described for classification for neurotoxicity and developmental neurotoxicity should be applied also to other target organ toxicities. The nervous system is well known for its long developmental period, which also continues after birth, and several nervous system functions can only be tested after a certain postnatal developmental age as a sufficient maturation stage must have been reached before a meaningful test can be performed. However, it is also recognised that the histological, biochemical and functional development of many other major organ systems including immune, sexual, hepatic, renal, respiratory, endocrine and cardiovascular systems proceed well into the postnatal period, even if the initial establishment of most organs is complete by the time when closure of the hard palate is complete (IPCS/EHC Document No 225, Principles For Evaluating Health Risks To Reproduction Associated With Exposure To Chemicals).

The arguments supporting the conclusion that this approach should be taken are given below.

1. According to CLP, developmental toxicity includes any effect interfering with normal development, resulting from exposure until sexual maturation

As given in section 3.7.1.4[1] of Annex I, classification for developmental toxicity is not limited to effects induced during pregnancy or due to parental exposure but includes also any effect interfering with normal development of the offspring resulting from exposure of the developing offspring to the time of sexual maturation, even if the classification is "primarily" intended as a hazard warning for pregnant women, and for men and women of reproductive capacity. It is also important to note that developmental effects can be manifested at any point in the life span of the organism.

[1] CLP 3.7.1.4. *Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation. However, it is considered that classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and for men and women of reproductive capacity. Therefore, for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure. These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency.*

The above interpretation of Annex I, section 3.7.1.4 of CLP is supported by the following reasons:

- The wording of that provision: the first sentence defines developmental toxicity. The second and third sentences explain the essence of this hazard class but "essentially" does not mean "exclusively" and should not be seen as a limitation to the definition of development toxicity in the first sentence.

- The same wording is used in the Commission's proposal for CLP (COM (2007) 355 final) and in the draft CLP Regulation agreed by the co-legislators (European Parliament and Council). There is nothing that would suggest that such reading would go against the legislator's intention.

- The wording of Annex I, section 3.7.1.4 of CLP appears to correspond to that of section 3.7.1.3 of GHS concerning adverse effect on the development of the offspring. Therefore, the argument that such reading of this CLP provision would run against the corresponding GHS provision cannot be supported

  GHS_Rev9E_0.pdf (unece.org)

- The CLP objective of a high level of human health protection. A nervous system under development may be more vulnerable than an adult nervous system.

RAC considered that classification for developmental toxicity was not limited to effects induced during pregnancy or as the result of parental exposure but covered also effects interfering with normal development that resulted from exposure of the developing offspring until sexual maturation. RAC highlighted that such developmental effects could include any target organ toxicities and were not limited to nervous system effects.

2. It is generally not possible to distinguish the precise origin or timing of the toxicological insult when the exposure has continued after the developmental period

As regards the exposure period of studies for developmental neurotoxicity, the offspring in an OECD TG 426 study are exposed when a substance is administered to the mothers daily as a minimum from the time of implantation (starting on gestation day (GD) 6) and throughout lactation (until postnatal day (PND) 21). The gestational and postnatal days specified in the OECD TG 426 are specific to commonly used strains of rats, as according to the TG the preferred test species is the rat. However, other species can be used when appropriate, and comparable gestational and postnatal days should be selected if a different species or unusual strain is used (please see further details in paragraph 7 of the OECD TG 426).

Cohort 2B of an extended one generation study in accordance with OECD TG 443 (EOGRTS) is terminated on PND 21 or 22 and therefore the offspring are exposed only *in-utero* via their mother and during the lactation period. In cohort 2A of a EOGRTS, the offspring are exposed via the mother *in utero*, through lactation and directly at least after weaning until termination on ~PND 66-77. It is to be noted that when exposure occurs via feed, there is also some direct exposure of the offspring via feed during the lactation period when the pups start eating the same feed as their mothers at around PND 10. In cohort 2A of EOGRTS, the auditory startle test is performed on PND 24 (±1 day) in this cohort and the functional observational battery and an automated test of motor activity including habituation between PND 63 and 75. However, it is generally not possible to distinguish the precise origin or timing of the toxicological insult if adverse neuropathological, functional, or behavioural outcome is observed after sexual maturation in cohort 2A.

RAC considers that the effects investigated or detected in Cohort 2B of EOGRTS and in the offspring in OECD TG 426 should be assessed and concluded under developmental toxicity. As clarified under point 1 above, classification for developmental toxicity is not limited to effects induced during pregnancy or as a result of parental exposure but covers also effects

interfering with normal development that result from exposure of the developing offspring until sexual maturation. Furthermore, if only effects caused by *in utero* exposure were considered relevant for the classification for developmental toxicity, rat studies could cover the neurodevelopmental processes only until a developmental stage that corresponds to that in human foetuses at gestation week 23 (see Annex I, below).

RAC considers that also the effects investigated after sexual maturation in cohort 2A of EOGRTS (and in the offspring in OECD TG 426 also if the exposure had continued after sexual maturation) should be addressed and concluded under developmental toxicity. According to the CLP criteria for developmental toxicity, developmental effects can be manifested at any time point of the life span of the organism, and in OECD TG 443 in which cohort 2A is exposed *in utero*, and postnatally until PND 66-77, or in any other study where the exposure has continued after the developmental period, it is not possible to know how much prenatal *in utero* exposure and/or postnatal developmental exposure until sexual maturation and/or exposure after sexual maturation of the offspring until PND 66-77 contributed to the manifestation of effects observed after sexual maturation. In addition, the cohort 2A of an EOGRTS and any design of OECD TG 426 has been designed to specifically investigate developmental neurotoxicity and effects on the nervous system caused by exposure at any stage of the developing nervous system and those seen in the offspring at any time point are of concern for developmental neurotoxicity. It is also a scientific fact that the nervous system continues to develop even after sexual maturation through adolescence (see Annex I).

RAC notes that a similar approach should also be applied to other target organ toxicities investigated at any point in the lifespan of the offspring that has been exposed during the developmental period (covering prenatal and postnatal period until sexual maturation as determined by preputial separation and vaginal opening), even if the exposure continued after sexual maturation.

3. Effects on or via lactation and developmental toxicity are assessed separately in their own right

Effects on or via lactation (Lact.) should not be used to replace the classification for developmental toxicity. Lactation is a separate classification category, and both hazard categories are assessed separately in their own right. Therefore, if a substance is e.g. neurotoxic due to developmental exposure (pre- and/or postnatal exposure until sexual maturation) and meets the CLP criteria for developmental toxicity, it should be classified for developmental toxicity. If there is also information that a substance posing such hazard can be present in breast milk in amounts sufficient to cause concern via lactation, then also a classification for effects on or via lactation should be assigned for a substance in addition to developmental toxicity classification. The users (lactating mothers) need to know that their breast-fed babies can be exposed to concerning amounts of developmental toxic substance also via milk when the lactating mothers themselves have been exposed to the substance. If the substance interferes with lactation by impacting adversely only the quantity or quality (nutrient composition) of milk, but the substance is not a developmental toxicant per se, then only a classification for effects on or via lactation and not for developmental toxicity can be warranted.

4. STOT RE and STOT SE do not cover effects that are specifically addressed under reproductive toxicity (see CLP 3.8.1.1 and 3.9.1.1)

According to CLP 3.9.1.1, specific target organ toxicity repeated exposure means specific toxic effects on target organs occurring after repeated exposure to a substance or mixture and includes all significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed, and which are not specifically addressed in sections 3.1 to 3.8 and 3.10. Developmental toxicity is addressed specifically in section 3.7 and includes any effect interfering with normal development such as death of the developing organism, structural abnormality, altered growth and functional deficiency (CLP 3.7.1.3). According to CLP criteria for developmental toxicity, other toxicity such as maternal toxicity shall be considered only with respect to its possible influence on the toxic effects on the developing offspring, and other toxicity can be used to dismiss the developmental effects from classification only if the developmental toxicity is solely considered to be a secondary non-specific consequence of other toxic effects. The CLP Regulation does not refer to higher sensitivity of offspring as compared to parental generation in the classification criteria for developmental toxicity. The term "double classification" is only discussed in CLP Guidance under Acute tox and STOT SE, stating "H*owever, care should be taken not to assign each class for the same effect, essentially giving a multiple classification, even where the criteria for different classes are fulfilled. In such a case the most appropriate (the most severe hazard) class should be assigned*."

RAC considers that developmental neurotoxicity investigated specifically e.g. in the DNT cohorts of an EOGRTS and in the offspring of an OECD TG 426 study should be addressed under developmental toxicity, independently of the potential neurotoxicity seen in adults and even if no sensitivity differences were observed between neurotoxic effects caused by developmental exposure and by exposure of mature nervous system. Higher sensitivity of the offspring to the neurotoxic effect as compared to the parental generation should not be what determines addressing DNT under developmental toxicity rather than under STOT, because this condition is not included in the CLP criteria for developmental toxicity. Often different types of tests are conducted in different studies (on adults and offspring), making potency comparisons difficult. In addition, the available tests for DNT and adult neurotoxicity may not be sensitive and exhaustive enough to always reveal the sensitivity and/or susceptibility differences between the parental generation and offspring.

However, other neurotoxicity, i.e. neurotoxicity that is caused by exposure of the mature nervous system to a substance, that is not specifically addressed under developmental toxicity or other hazard class (the provision relating to STOT RE of effects which are not specifically addressed in sections 3.1 to 3.8 and 3.10 and to STOT SE of effects which are not specifically addressed in sections 3.1 to 3.7 and 3.9 to 3.10 needs to be taken into account), should be addressed under STOT SE or RE, depending on the length of exposure required to cause such effects, and such effects may lead to classification as STOT RE/SE. If the CLP criteria for both developmental toxicity and STOT RE are met from studies relevant for addressing classification in the respective hazard classes, classification in both hazard classes should be applied and it is not to be considered as a "double classification".

5. OECD test guidelines 426 and 443 (cohort 2A and 2B) designed to specifically assess developmental neurotoxicity

The cohorts 2A and 2B of an EOGRTS as well as an OECD TG 426 study have been designed to specifically investigate developmental neurotoxicity and effects on the nervous system

caused by exposure at any stage of the developing nervous system and seen in the offspring at any time point are of concern for developmental neurotoxicity.

RAC considers that using an OECD test guideline designed to assess developmental neurotoxicity for assessing "adult" neurotoxicity instead of developmental neurotoxicity would question the validity and purpose of these test methods, which are under the OECD Mutual Acceptance of Data (MAD) system.

An agreed approach

RAC agreed that neurotoxic effects investigated or detected at any point in the life span of the organism that had been exposed during the developmental period, covering both prenatal and postnatal developmental period until sexual maturation (determined in rats by preputial separation in males and vaginal opening in females), even if the exposure had continued also after sexual maturation, should be assessed and concluded under developmental toxicity whereas neurotoxic effects caused by exposure of mature animals/humans (exposure only after sexual maturation) should be assessed and concluded under STOT SE or RE as follows:

Type of investigation (with or without positive findings)	**Related hazard class to assess and conclude on classification**	**Remarks**
Neurotoxicity in offspring (animals or humans) at any point in the life span exposed during developmental period, covering both prenatal and postnatal developmental period until sexual maturation, even if the exposure continued also after sexual maturation	Repr. (development)	Specifically investigated e.g. in cohort 2A and 2B in EOGRTS, and in offspring in OECD TG 426
Neurotoxicity in animals or humans exposed at mature stage (exposure only after sexual maturation)	STOT SE or RE	Investigated e.g. in OECD TG 424 and to limited extent in OECD TG 408, 407 and acute toxicity studies, in P0 generation in EOGRTS and OECD TG 421/422.*
Neurotoxicity in offspring (animals or humans) at any point in the life span exposed during developmental period, covering both prenatal and postnatal developmental period until sexual maturation, even if the exposure continued also after sexual maturation	Repr. (development) **and** STOT SE or STOT RE, respectively	

and Neurotoxicity in animals or humans exposed at mature stage (exposure only after sexual maturation)		

* According to these OECD TGs, young healthy adult animals should be employed in these studies, and normally and historically the dosing has started only after sexual maturation as determined by preputial separation and vaginal opening in males and females, respectively. However, in some cases the exposure may have started before sexual maturation as e.g. the OECD TG 407 and 408 are flexible with that respect: "Dosing should begin as soon as feasible after weaning, and, in any case, before the animals are nine weeks old." If the exposure has started very soon after weaning and the animals have not achieved their sexual maturation, the observed effects in these animals may be of developmental origin and assessed and concluded as such.

RAC highlights that neurotoxicity and developmental neurotoxicity are used as important examples in this paper, but a similar approach should be applied also to other target organ toxicities.

Annex I

Development of the nervous system continues after weaning and the developing nervous system is especially vulnerable to neurotoxic insults

It has been recognised that the developing nervous system is especially vulnerable to certain chemicals, and exposures may result in altered neural development with consequences that may be quite unlike the chemical's effects in an adult nervous system (see also North American Free Trade Agreement (NAFTA) Technical Working Group on Pesticides (TWG) Developmental Neurotoxicity Study Guidance Document December, 2016).

Development of the nervous system involves a complex interplay between multiple developmental processes that occur both prenatally and postnatally and are spatially and temporally regulated. In CLP developmental toxicity refers to effects interfering with normal development caused by exposure until sexual maturation, but development of the nervous system differs from most other organs, as it continues to develop even after sexual maturation through adolescence, reaching adult levels of neurotransmitters, synaptic plasticity, myelination and grey matter at around age of 20 in humans and around PND60 in rats. The developmental status of a new-born rat's nervous system corresponds to approximately that of a preterm human infant (gestation week 23), while PND 21 in rats corresponds approximately to a 3-year old human child (the figure below adapted from Semple et al., 2013; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3737272/). It is important to note that this interspecies comparison of developmental timelines should be interpreted with care, as it is based primarily on major neurodevelopmental events/phases and does not necessarily take into account many of the less pronounced processes (e.g. various subcellular events). In addition, the inter-individual variation regarding neurodevelopmental timing further introduces uncertainty behind the interspecies comparison.

Human	Rodent	Developmental milestones
23–32 wk gestation (pre-term infant)	pnd 1–3	Oligodendrocyte maturation state changes—predominance of mitotically active pre-OLs[a]. Immune system development. Establishment of the blood-brain barrier.
36–40 wk gestation (term infant)	pnd 7–10	Peak brain growth spurt. Peak in gliogenesis. Increasing axonal and dendritic density. Oligodendrocyte maturation state changes–switch to a pre-dominance of immature OLs. Consolidation of the immune system.
2–3 year old	pnd 20–21	Brain reaches 90–95% of adult weight. Peak in synaptic density at 50%> adult levels. Peak in myelination rate. Neurotransmitter and receptor changes.
4–11 year old	pnd 25–35	Fractionation/specialization of prefrontal cortex neural networks (structural maturation). Maximum volume of grey matter and cortical thickness.
12–18 year old	pnd 35–49	Reduced synapse density, reaching a plateau at adult levels. Refinement of cognitive-dependent circuitry. Ongoing myelination; increasing white matter volume and fractional anisotrophy.
20 years +	pnd 60+	Adult levels of neurotransmitters. Adult levels of synaptic density. Ongoing myelination and declining grey matter.

Annex II

DNT under some other regulations

Under REACH adult neurotoxicity is used as a trigger for requesting developmental neurotoxicity cohorts 2A and 2B in EOGRTS, and STOT SE/RE classification is not an adaptation criteria to this requirement

DNT cohorts in EOGRTS are not a standard data requirement under REACH when EOGRTS is required, but are triggered under REACH Annex IX and Annex X in case particular concerns on (developmental) neurotoxicity are identified. Recognised triggers include e.g. functional or morphological alterations observed in mature nervous system. Adult neurotoxicity is thereby a particular concern for developmental neurotoxicity and specifically investigated by tests on developmental neurotoxicity (such as cohorts 2A and 2B in EOGRTS) if the substance is known to cause adult neurotoxicity.

In the Biocidal Products Regulation (BPR) developmental neurotoxicity is placed under Reproductive toxicity

Section 8.10.3 of BPR covers developmental neurotoxicity and it is a subsection of Reproductive toxicity (8.10). According to BPR 8.10.3, information on developmental neurotoxicity may be produced according to OECD TG 426, or any relevant study set providing equivalent information, or by Cohorts 2A and 2B of an EOGRTS (OECD TG 443) with additional investigation for cognitive functions.